American Journal of Hematology 54:1–11 (1997)

Lineage- and Differentiation Stage-Specific Expression of LSM-1 (LPAP), a Possible Substrate for CD45, in Human Hematopoietic Cells

Y. Shimizu,[1] H. Sugiyama,[2]* Y. Fujii,[3] K. Sasaki,[1] K. Inoue,[1] H. Ogawa,[1] H. Tamaki,[1] S. Miyake,[1] Y. Oji,[1] T. Soma,[1] T. Yamagami,[1] M. Hirata,[1] K. Ikeda,[1] T. Monden[4] and T. Kishimoto[1]

[1]Department of Medicine III, Osaka University Medical School, Osaka, Japan
[2]Department of Clinical Laboratory Science, Osaka University Medical School, Osaka, Japan
[3]First Department of Surgery, Osaka University Medical School, Osaka, Japan
[4]Second Department of Surgery, Osaka University Medical School, Osaka, Japan

CD45, a transmembrane tyrosine phosphatase, is found on almost all nucleated hematopoietic cells and plays a crucial role in lymphocyte activation and differentiation. We recently achieved isolation of the human LSM-1 (hLSM-1) gene, whose product is a possible substrate for CD45, and we raised antibodies against the hLSM-1 protein. hLSM-1 expression in hematopoietic cells was examined with Northern and Western blot, fluorescence-activated cell sorter, and immunocytochemical analyses. It was found that in the lymphoid lineage, T and B lymphocytes as well as NK cells expressed LSM-1, whereas terminally differentiated plasma cells did not. As for the myeloid lineage, immature myeloid cells expressed LSM-1, whereas terminally differentiated granulocytes and monocytes did not. In the erythroid lineage, normal erythroblasts expressed very low levels of LSM-1, while erythroid cell lines (K562 and HEL) did not. Megakaryocytes did not express LSM-1. Both $CD34^+/CD33^-$ and $CD34^+/CD33^+$ hematopoietic progenitor cells weakly expressed LSM-1. These results showed that LSM-1 is expressed in a lineage- and differentiation stage-specific fashion. Am. J. Hematol. 54:1–11, 1997

Key words: LSM-1; LPAP; CD45; hematopoietic cells

INTRODUCTION

CD45, a transmembrane tyrosine phosphatase, is found on all nucleated hematopoietic cells except for erythroid cells and is required for T-cell receptor (TCR) signal transduction.[1–5] Multiple CD45 isoforms arise from alternatively spliced exons of a single copy gene.[1] Three distinct exons, designated A, B, and C, generate the various known isoforms, which have been termed CD45RA, -RB, -RC, and -RO, respectively; these isoforms are expressed in a tissue-specific, activation-specific, and developmental manner, suggesting an important function for this extracellular domain. CD45 isoforms on lymphocytes are expressed in a lineage- and activation-specific manner. Mature B cells express only the highest molecular weight isoform of CD45, B220 (exon A^+, B^+, C^+), whereas mature T cells express multiple isoforms, CD45RA (exon A^+), -RB (exon B^+), -RC (exon C^+), and -RO (exon A^-, B^-, C^-).[2] Furthermore, developmental regulation of CD45 isoform expression is believed to occur in the T-cell population. Mature thymocytes predominantly express the CD45RO isoform, whereas peripheral blood T cells express either the CD45RA or CD45RO isoform.[6] In addition to the developmental regulation, CD45 on T cells alters its isoforms following activation.

The intracellular domains of all CD45 molecules possess intrinsic protein tyrosine phosphatase activity and are highly conserved among different species, suggesting a crucial role of CD45 in lymphocyte activation and differentiation.[3] The most direct evidence for the involvement of CD45 in regulating TCR signal transduction has

*Correspondence to: Haruo Sugiyama, Departments of Medicine III and of Clinical Laboratory Science, Osaka University Medical School, 2-2, Yamada-Oka, Suita City, Osaka 565, Japan.

Received 12 December 1995; Accepted 7 August 1996

come from the finding that CD45-deficient mouse T-cell clones fail to proliferate or produce cytokines in response to antigen or anti-TCR monoclonal antibody.[5,7,8] Similarly, CD45-deficient cytotoxic T cells are unable to lyse target cells.

Possible candidates for physiological substrates of CD45 in T cells are two members for the *src* family of protein tyrosine kinase (PTK), $p56^{lck}$ and $p59^{fyn}$, both of which appear to be crucial for optimal T-cell activation and development.[9–12] These PTKs possess a C-terminal tyrosine that, when phosphorylated, downregulates enzymatic activity. Since CD45 has been shown to be capable of dephosphorylating both the regulatory tyrosine 505 of $p56^{lck}$ as well as tyrosine 531 of $p59^{fyn}$ in vitro, one postulated role for CD45 is to dephosphorylate these C-terminal tyrosines, enabling the PTK to become activated after the appropriate receptor is bound.

With the aid of subtraction hybridization between mouse pre-B and myeloma cells, we recently succeeded in isolating the lymphocyte-specific molecule-1 (mLSM-1) gene that is expressed in mouse lymphocytes but not in mouse plasma cells.[13] At the same time, others have purified the CD45-associated proteins from mouse and human T-cell lines, isolated its cDNA clones, and named them CD45-AP (CD45-associated 30-kDa phosphorylated protein) in mouse[14] and lymphocyte phosphatase-associated phosphoprotein (LPAP) in human.[15] LPAP is thought to be a possible candidate for a physiological substrate of CD45 on the basis of the following findings: 1) LPAP is coexpressed and physically associates with CD45; and 2) in pervanadate-treated human T lymphocytes, LPAP undergoes phosphorylation on tyrosine residues in vivo, whereas in T lymphocytes expressing enzymatically active CD45, tyrosine phosphorylation of LPAP is undetectable. It was found that mLSM-1 was identical to CD45-AP and that LPAP was a human homologue of mLSM-1/CD45-AP. To elucidate further the role of LSM-1 in human hematopoietic cells, we isolated a human homologue (hLSM-1) of mLSM-1 and raised antibodies against the hLSM-1 protein. We describe here lineage- and differentiation stage-specific expression of hLSM-1 in human hematopoietic cells and discuss the roles of hLSM-1 in the proliferation and/or differentiation of hematopoietic cells.

MATERIALS AND METHODS

Cells

Human T-cell acute lymphoblastic leukemia cell lines (TALL-1, Jurkat), a human adult T-cell leukemia cell line (MT-1), human B lymphoblast cell lines (BALL-1, Daudi), and a human myeloma cell line (RPMI 8226) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, and 5×10^{-5} M 2-mercaptoethanol. A human promyelocytic leukemia cell line (HL-60), human monocytic cell lines (U937 and J111), a human erythroid cell line (k562) derived from chronic myelogenous leukemia blast crisis, and a human erythroleukemia cell line (HEL) were cultured in the same medium as described above but without 2-mercaptoethanol.

Isolation of Human LSM-1 cDNA

A λgt10 cDNA library was constructed from poly $(A)^+$ RNA of TALL-1 using a cDNA synthesis kit (Amersham, Little Chalfont, UK). With the mLSM-1 gene used as a DNA probe, plaque hybridization was performed in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 1% sodium dodecyl sulfate (SDS), and 200 μg/ml denatured salmon sperm DNA at 42°C. As a result, five positive clones were isolated from 5×10^5 independent clones. The cDNA inserts were then subcloned into a plasmid vector and sequenced with the dideoxy chain termination method, resulting in the isolation of hLSM-1, a human homologue of mLSM-1. The nucleotide sequence of hLSM-1 was identical with that of LPAP.[14]

Northern Blot Analysis

Thirty micrograms of total RNA from fresh leukemia cells and various cell lines were applied to 1.2% agarose gels containing 6.7% formaldehyde and electrophoresed. RNA was transferred to a nylon membrane filter in 20 × SSC. Hybridization was performed at 42°C with the hLSM-1 DNA probe labeled with ^{32}P in 50% formamide, 5 × SSPE, 10 × Denhardt's solution, 2% SDS, and 100 μg/ml denatured salmon sperm DNA. The filter was first washed in 2 × SSC and 0.05% SDS at room temperature for 40 min and then in 0.1 × SSC and 0.1% SDS at 65°C for 40 min, and autoradiographed. Northern blot analysis of various human tissues was performed with Multiple Tissue Northern Blot Kits (Clontec Laboratories, CA, USA) according to the manufacturer's directions.

Anti-hLSM-1 Antibodies

Escherichia coli, BL21 was transfected with the plasmid vector pGEX-4T of the GST Gene Fusion System (Pharmacia Biotech, Uppsala, Sweden), into which the coding region of the cytoplasmic portion of the hLSM-1 had been inserted, and grown at 37°C overnight and then in the presence of 1 mM IPTG at 37°C for 4 hr. Cells were harvested, suspended at a final concentration of 6% in buffer A (50 mM Tris-HCl [pH 8.0], 0.5 mM EDTA, 0.4 M NaCl, 5 mM $MgCl_2$, 5% glycerol, 0.1 mM PMSF, and 0.1 mM DTT) containing 1 mg/ml lysozyme, incubated on ice for 1 hr, and freeze-thawed twice. EDTA and NP-40 were added to the cell suspension to final concentrations of 1 mM and 0.5% (vol/vol), respectively. The cell suspension was then sonicated and centrifuged at 15,000 rpm for 15 min. Glutathione sepharose 4B was added to the supernatant, and the mixture was rotated at 4°C for 30 min. After centrifugation, the pellet was washed with buffer A containing 1 mM EDTA and 0.5%

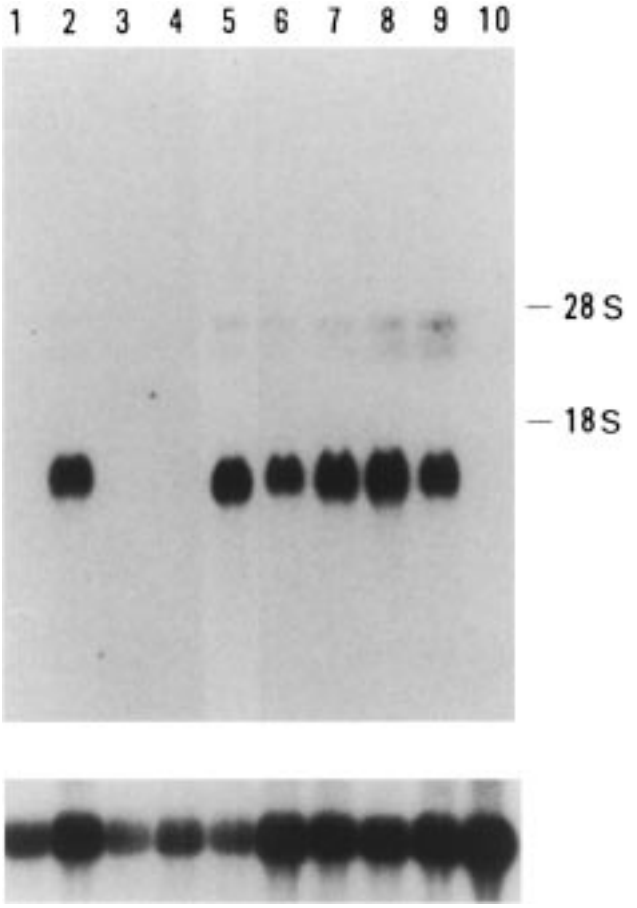

Fig. 1. Northern blot analysis of hematopoietic cell lines for hLSM-1 expression. RNA from various cell lines was electrophoresed on 1.2% agarose gels and then blotted onto a nylon membrane filter. The filter was hybridized with ^{32}P-labeled hLSM-1 DNA probe and autoradiographed. Representative cell lines are shown here. Lane 1, K562; lane 2, HL-60; lane 3, J111; lane 4, HEL; lane 5, TALL-1; lane 6, Jurkat; lane 7, MT-1; lane 8, Daudi; lane 9, BALL-1; lane 10, RPMI 8226.

and the fusion proteins were eluted by the addition of 10 mM reduced glutathione. Rabbits were immunized with the fusion proteins by standard methods. The anti-hLSM-1 antibodies were purified with a column that was coupled with the hLSM-1 cytoplasmic portion protein prepared by thrombin cleavage of the fusion proteins. The antibodies were then used for the experiments.

Western Blot Analysis

Western blot analysis was performed as described previously.[16] Cells were washed with phosphate-buffered saline (PBS) and lysed with Laemli's sample buffer. The cell lysates from 2×10^5 cells were boiled for 5 min and applied to the lanes in 12.5% SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred to Immobilon polyvinylidene difluoride (Millipore, MA, USA). The filter was probed with anti-hLSM-1 antibodies and then with horseradish alkaline phosphatase-linked

TABLE I. Distribution of hLSM-1 Expression

	Normal blood cells					Cell lines and fresh leukemia cells				
hLSM-1 expression	Stem and progenitor cells	Lymphoid	Myeloid	Erythroid	Megakaryocytes	Lymphoid	Myeloid	Erythroid	Megakaryocytes	Organs
Positive										
Strong		T lymphocytes	Myelocytes			TALL-1	HL-60			Spleen
			Metamyelocytes			Jurkat	KG-1			Thymus
						LC4-1				Peripheral blood lymphocytes
						MT-1				Small intestine
		B lymphocytes				BALL-1				Colon
						Daudi				Lung
						ALL-L_2	AML-M_3			
						ALL-L_2				
Weak	$CD34^+/CD33^-$ cells		Granulocyte (stab)				CML-CP			Prostate
	$CD34^+/CD33^+$ cells									Ovary
										Heart
										Liver
										Skeletal muscle
										Pancreas
Negative or below detectable levels			Granulocytes (stab, segmented)			RPMI 8226	U937	K562		Testis
			Monocytes				J111	HEL		Brain
										Placenta

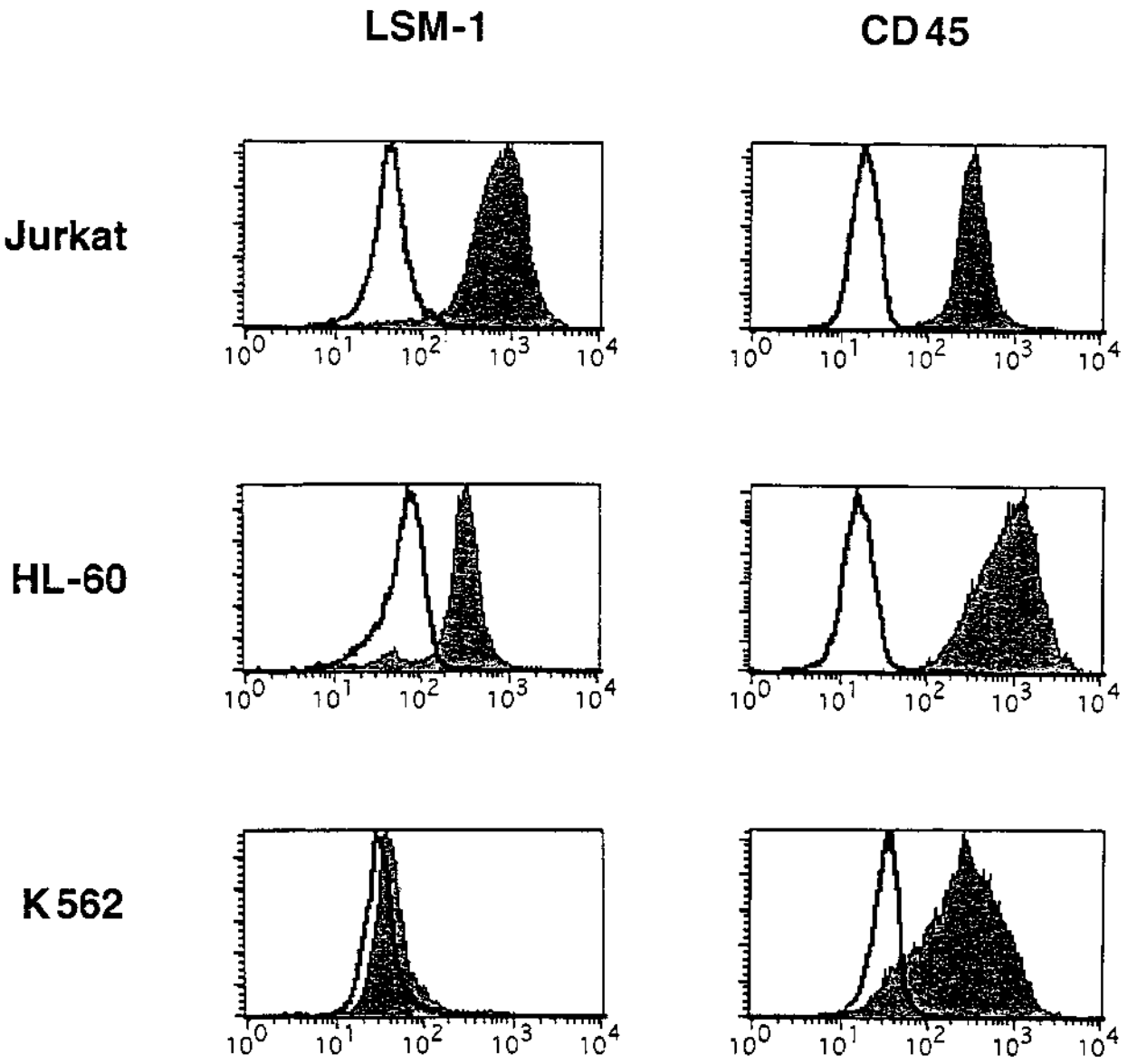

Fig. 2. FACS analysis of a T-cell line and myeloid and erythroid cell lines for LSM-1 expression. Exponentially growing Jurkat (T-cell), HL-60 (myeloid), and K562 (erythroid) cell lines were FACS analyzed for the expression of LSM-1 and CD45. Shaded histograms indicate LSM-1 or CD45 expression. Open histograms are for the control stained cells (normal rabbit immunoglobulin for LSM-1 and isotype-matched FITC-labeled mouse monoclonal antibody with irrelevant specificity for CD45).

anti-immunoglobulin antibodies (Amersham, Little Chalfont, UK). It was then washed and next immersed in detection reagents (Amersham) for 1 min and autoradiographed for 1–5 min. After two washings with TBST (Tris-buffered saline containing 0.05% Tween 20), the same filter was probed with anti-actin monoclonal antibody (Oncogene, NY, USA), processed as described above, and then autoradiographed.

FACS Analysis

Fluorescein isothiocyanate (FITC)-conjugated anti-CD45 (common epitope), PE-conjugated anti-CD19, -CD33, and -glycophorin A, and biotin-conjugated anti-CD34, -CD19, and -CD38 were purchased from Dakopats (Sweden). FITC-conjugated anti-Leu7, PE-conjugated anti-CD3, CD16, and biotin-conjugated anti-CD3 were purchased from Becton-Dickinson (Mountain View, CA, USA). FITC-conjugated anti-LFA-1 (CD11a) was obtained from Immunotech (Marseille, France).

Samples for fluorescence-activated cell sorter (FACS) analysis of tonsil lymphocytes were prepared as follows. A single cell suspension was prepared by mincing the tonsils with a pair of scissors and pressing the pieces through a stainless steel mesh, after which the mononu-

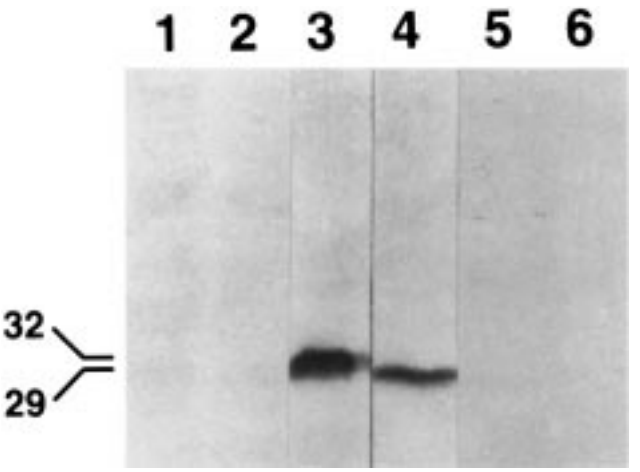

Fig. 3. Western blot analysis of various cell lines for LSM-1 expression. Cell lysates were electrophoresed on 12.5% SDS-polyacrylamide gels, and the proteins were blotted to filters. The filters were probed with anti-hLSM-1 antibodies (lanes 1–4) or with anti-hLSM-1 antibodies that were absorbed with hLSM-1 protein (lanes 5 and 6). Lane 1, U937; lane 2, K562; lanes 3 and 5, Jurkat; lanes 4 and 6, HL-60.

clear cells were separated with the Ficoll-Hypaque density gradient method and stored in liquid nitrogen until use. For FACS analysis of NK cells and monocytes, we used the mononuclear cells that were separated from peripheral blood by the Ficoll-Hypaque density gradient method. For FACS analysis of granulocytes, the buffy coat obtained by centrifugation of heparinized peripheral blood was put on Ficoll-Hypaque solution and centrifuged. The cells that were sedimented through a Ficoll-Hypaque layer were used for FACS analysis after lysing of the contaminated red blood cells. For FACS analysis of erythroblasts and hematopoietic progenitor cells, we used mononuclear cells that were separated from normal bone marrow cells with the Ficoll-Hypaque density gradient method.

Three-color flow cytometry was performed as described previously with minor modifications.[17] Two million cells were first stained with a combination of PE- and biotin-conjugated monoclonal antibodies. The cells were washed and then stained with FITC-conjugated monoclonal antibodies and streptavidin-conjugated red 670 (GIBCO, Gaithersburg, MD, USA). For staining of the cytoplasmic portions of the LSM-proteins, the cells that had been stained with a combination of PE- and biotin-conjugated monoclonal antibodies were fixed with 0.1% formalin at room temperature for 30 min and then permeabilized by treatment with 0.05–0.1% saponin (Sigma, St. Louis, MO, USA). The cells were again washed and then reacted with 10 μg/ml of anti-hLSM-1 antibodies or the same concentration of normal rabbit immunoglobulin, followed by reaction with a combination of FITC-conjugated goat anti-rabbit antibodies [$F(ab')^2$](Dako) and streptavidine-conjugated red 670. Data from 500,000 events were usually obtained and analyzed with Cell Quest software (Becton Dickinson). Subsets of cells were gated as shown in the contour plots, and the cells in these gates were analyzed for the LSM-1 or CD45 expression. Control histograms are shown in blank, and the cut-off levels were set so that 98–99% of the control stained cells fall within the negative range.

Immunocytochemical Analysis

Immunocytochemical analysis of bone marrow cells was performed as described previously with minor modifications.[18] Mononuclear bone marrow cells from normal volunteers were applied to glass slides with Auto smear CF-12D (Sakura, Tokyo, Japan) and air dried. Cytospin specimens were rapidly fixed with microwave irradiation as described previously.[18] The specimens were first treated with 0.3% hydrogen peroxide in absolute methanol to inactivate endogenous peroxidase activity and then with 3% normal goat serum at room temperature for 15 min to block the nonspecific binding of antibodies. The cytospin specimens were washed with PBS and incubated at 4°C overnight with anti-hLSM-1 antibodies. They were washed with PBS, overlaid with biotinylated goat anti-rabbit IgG, and incubated at room temperature for 20 min. Finally, the specimens were incubated with peroxidase-labeled streptavidin for 20 min, and then specific immunostain was visualized after incubation of 3–7 min with reaction buffer containing 0.05 M Tris-HCL (pH 7.6), 0.002% hydrogen peroxide, and 0.02%, 3,3′-diaminobenzidine tetrahydrochloride.

RESULTS

Distribution of LSM-1 Expression in Human Hematopoietic Cell Lines and Fresh Leukemia Cells

LSM-1 expression in various kinds of human hematopoietic cell lines was examined by Northern blot analysis (Fig. 1 and Table I). LSM-1 was expressed in T-cell (TALL-1, Jurkat, LC4-1, and MT-1) and B-cell lines (BALL-1 and Daudi) and in myeloid cell lines (KG-1 and HL-60), but not in the myeloma cell line (RPMI 8226), the monocyte-like cell line (U937), the histiocytic cell line (J111), or two human erythroid cell lines (K562 and HEL). Freshly isolated leukemic cells were also examined for LSM-1 expression. LSM-1 was expressed in both of two acute lymphocytic leukemia (ALL)-L_2 samples, one of one acute myelocytic leukemia (AML)-M_3 sample, and one of one chronic myelocytic leukemia (CML) chronic phase sample, but not in the AML-M_5 sample.

These cell lines were analyzed for hLSM-1 expression by FACS using anti-hLSM-1 antibodies. The same results as those for Northern blot analysis were obtained. (The patterns of FACS analysis of representative cell lines are shown in Figure 2.)

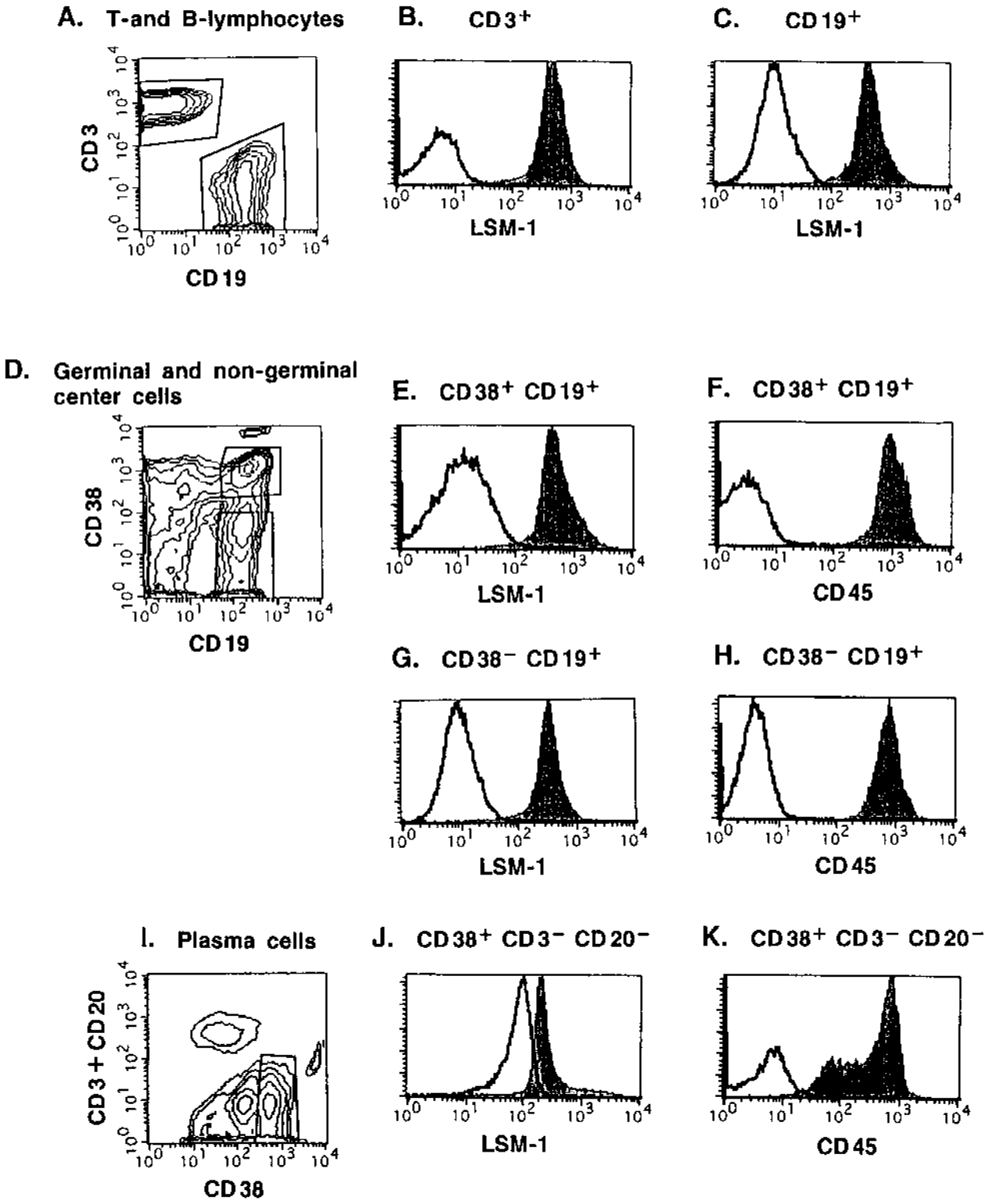

Fig. 4. Strong expression of LSM-1 in mature T and B lymphocytes, but no expression of LSM-1 in plasma cells. A–C: Three-color flow cytometric analysis (LSM-1 CD3/CD19) of the mature T and B lymphocytes from tonsils. D–H: Three-color flow cytometric analysis (LSM-1 or CD45/CD19/CD38) of the B lymphocytes from tonsils. Germinal ($CD38^+/CD19^+$) and non-germinal ($CD38^-/CD19^+$) center cells were FACS analyzed for the expression of LSM-1 and CD45. I–K: The plasma cell fraction ($CD38^+/CD3^-/CD20^-$) in bone marrow was FACS analyzed for the expression of LSM-1 and CD45. Shaded histograms indicate LSM-1 or CD45 expression; open histograms indicate control stained cells.

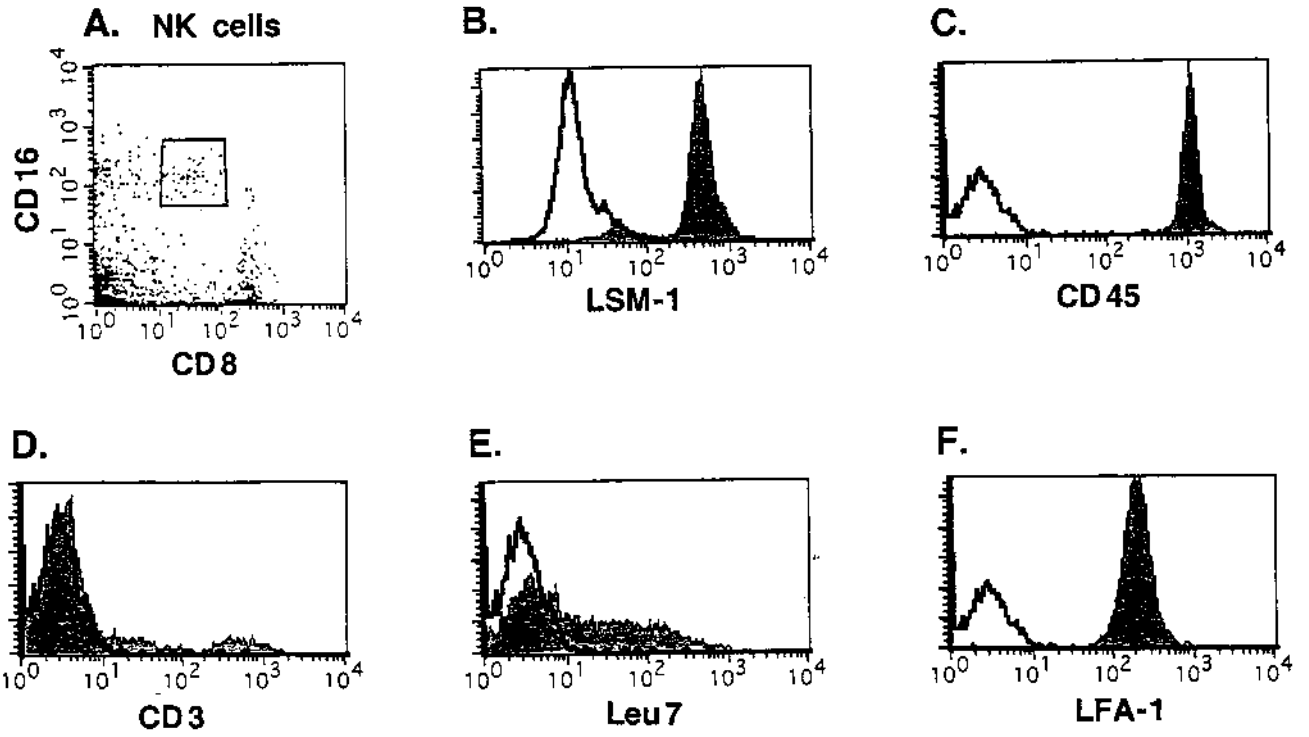

Fig. 5. Strong expression of LSM-1 in NK cells. A–C: NK cells ($CD16^+/CD8^{low}$) were FACS analyzed for the expression of LSM-1 and CD45. D–F: The NK cell fraction was FACS analyzed for the expression of CD3, Leu7, and LFA-1.

Western Blot Analysis for LSM-1 Expression

To confirm LSM-1 expression at protein levels, the cell lysates of various cell lines were examined for the LSM-1 protein by Western blot analysis (Fig. 3). This protein was detected in Jurkat and HL-60, but not in U937 or K562. In Jurkat, two different forms of 29 and 32 kDa were detected, as previously described, but in HL-60 and KG-1 only the 32 kDa form was detected.[15] These results confirmed the findings obtained by Northern blot and FACS analysis. With the anti-hLSM-1 antibodies that were absorbed with the hLSM-1 proteins, Western blot analysis could not detect the LSM-1 protein, confirming the specificity of anti-hLSM-1 antibodies against LSM-1 proteins.

LSM-1 Expression in Normal Hematopoietic Cells

T and B lymphocytes in tonsils were analyzed for LSM-1 expression by three-color flow cytometry using the antibodies against LSM-1, CD3, and CD19 in combination with FITC/PE/red 670 fluorescence (Fig. 4). T and B lymphocytes strongly expressed both LSM-1 (Fig. 4A–C) and CD45 (data not shown). Any difference in LSM-1 expression between germinal ($CD38^+/CD19^+$) and nongerminal ($CD38^-/CD19^+$) center B cells was also examined (Fig. 4D–H), but with negative results, since both populations strongly expressed both LSM-1 and CD45. Plasma cells ($CD38^+/CD3^-/CD20^-$) in bone marrow either did not express LSM-1 or expressed it at very low levels (Fig. 4I–K).[19] However, natural killer (NK) cells ($CD16^+/CD8^{low}$) in peripheral blood expressed both LSM-1 and CD45 (Fig. 5), while surface markers of this gated fraction confirmed the characteristics of NK cells, such as $CD3^-$/ $Leu7^+/LFA\text{-}1^+$.

Granulocytes and monocytes of peripheral blood strongly expressed CD45 but not LSM-1 (Fig. 6).

By using glycophorin A as a marker for erythroblasts, erythroblasts in bone marrow were analyzed for the LSM-1 expression (Fig. 7). These cells were found to express very low levels of LSM-1 and were negative for CD45 expression.

LSM-1 expression in megakaryocytes was immunocytochemically examined. Bone marrow cells on a smear were stained with anti-hLSM-1 antibodies. Lymphocytes, myeloblasts, and promyelocytes were stained, whereas megakaryocytes were not. Thus it appears that megakaryocytes do not express LSM-1.

Low Levels of Expression of LSM-1 in Both $CD34^+/CD33^-$ and $CD34^+/CD33^-$ Hematopoietic Progenitor Cells

Whether hematopoietic progenitor cells express LSM-1 was determined by three-color flow cytometry (LSM-

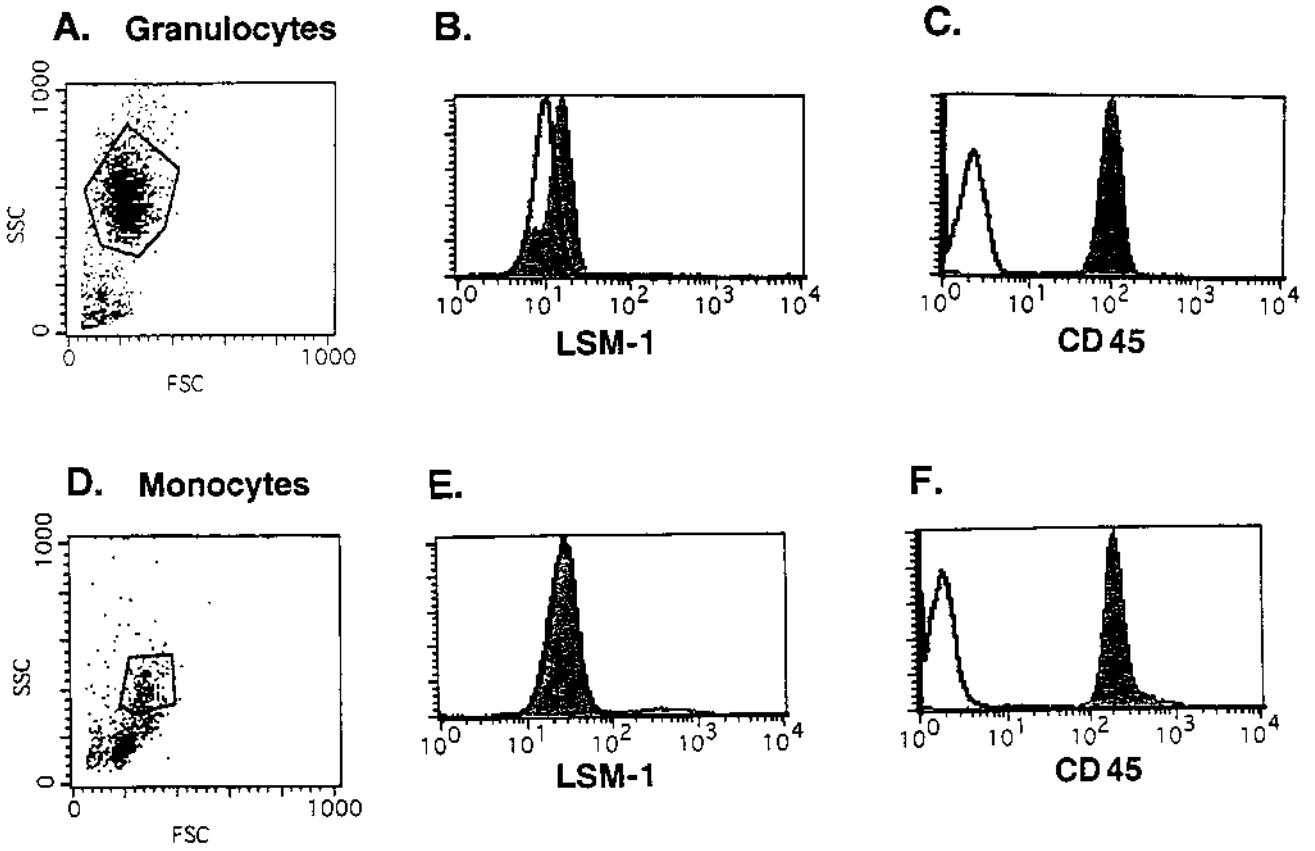

Fig. 6. No expression of LSM-1 in granulocytes and monocytes. A–C: The granulocyte fraction was gated accordig to the forward/side scatter pattern and examined for LSM-1 and CD45 expression. D–F: The Monocyte fraction was gated according to the forward/side scatter pattern and examined for LSM-1 and CD45 expression.

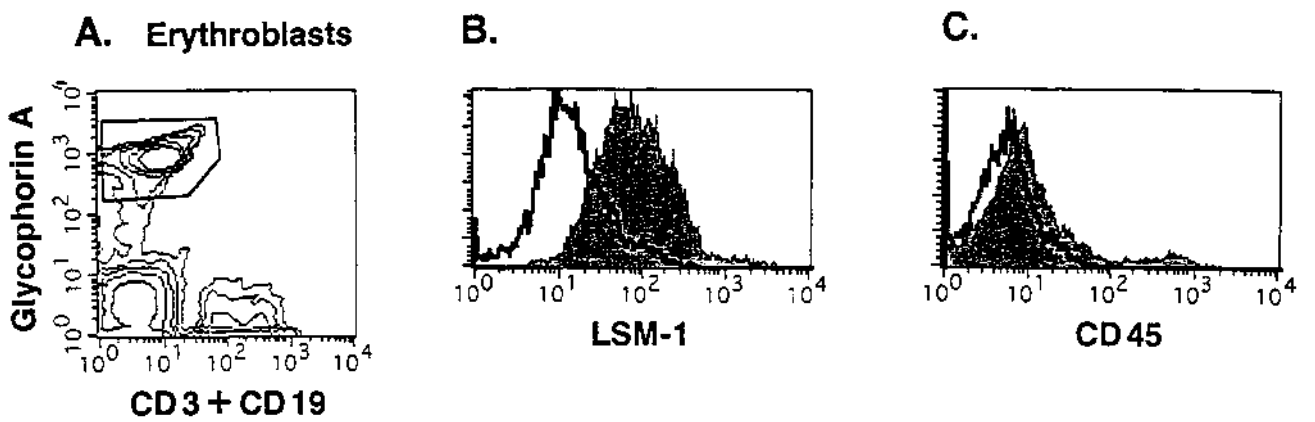

Fig. 7. A–C: Expression of LSM-1 in erythroblasts. The cells gated as glycophorin A^+/$CD3^-$/$CD19^-$ were FACS analyzed for LSM-1 expression. LSM-1 expression in erythroblasts was very low, and as few as 3% of glycophorin A-positive cells were stained above the cut-off levels (see Materials and Methods).

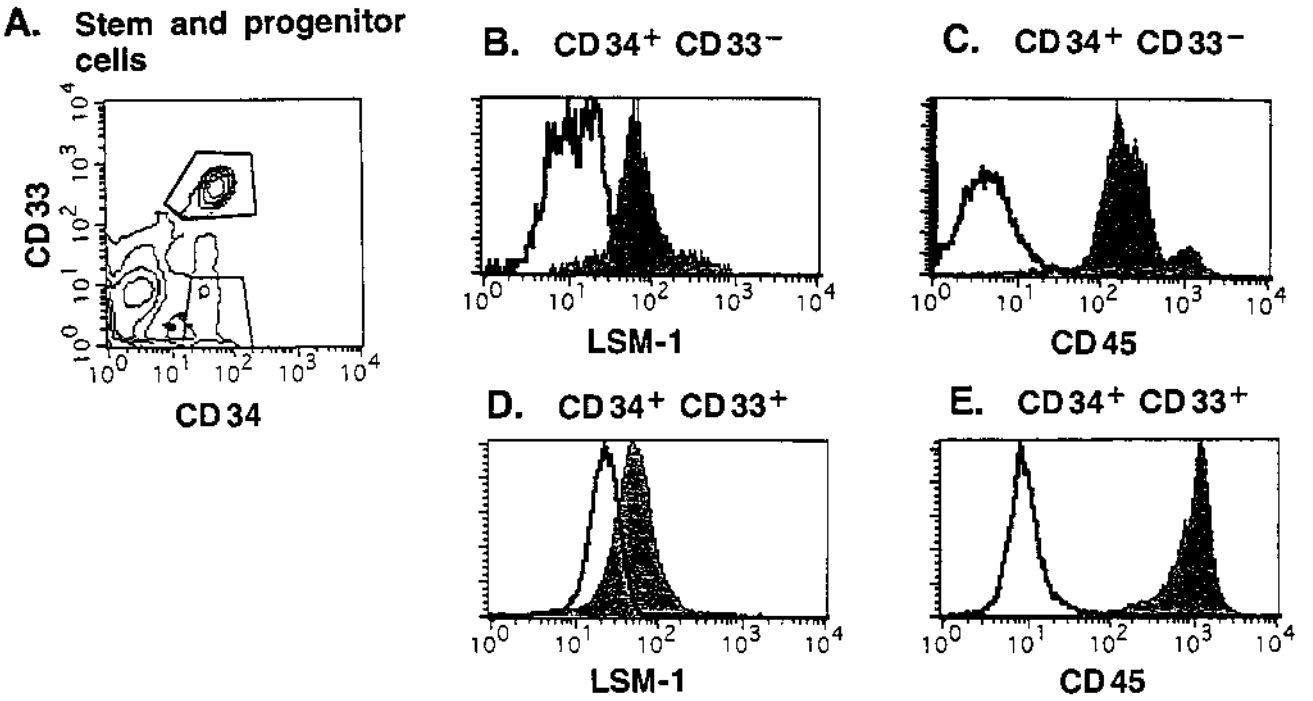

Fig. 8. A–E: Weak expression of LSM-1 in hematopoietic progenitor cells. Two gated fractions ($CD34^+/CD33^-$ and $CD34^+/CD33^+$) were FACS analyzed for LSM-1 and CD45 expression.

1/CD34/CD33) (Fig. 8). Both $CD34^+/CD33^-$ and $CD34^+/CD33^+$ hematopoietic progenitor cells weakly expressed LSM-1, while CD45 expression was intermediate in $CD34^+/CD33^-$ cells and strong in $CD34^+/CD33^+$ cells.

Tissue Distribution of LSM-1

LSM-1 expression was examined in various organs by Northern blot analysis (Fig. 9). LSM-1 was strongly expressed in spleen, thymus, peripheral blood lymphocytes, small intestine, and lung and weakly expressed in prostate, ovary, heart, liver, skeletal muscle, and pancreas. However, LSM-1 was not detected in testis, placenta, kidney, or brain.

DISCUSSION

LSM-1 expression was studied in human hematopoietic cells by means of Northern and Western blot analysis, FACS analysis, and immunocytochemical analysis; the results are summarized in Table I.

The expression pattern of the LSM-1 in hematopoietic cells can be summarized as follows: 1) among lymphoid lineage cells, mature T and B lymphocytes as well as NK cells express LSM-1, whereas terminally differentiated plasma cells do not; 2) as for myeloid lineage cells, immature myeloid cells (HL-60 and KG-1) express the LSM-1, but myeloid cells that have differentiated terminally to granulocytes and monocytes do not; 3) in the erythroid lineage, erythroblasts expressed only very low levels of LSM-1, but erythroid cell lines (K562 and HEL) did not express it at all; 4) megakaryocytes do not express LSM-1; and 5) both $CD34^+/CD33^-$ and $CD34^+/CD33^+$ hematopoietic progenitor cells weakly express LSM-1. Thus, LSM-1 is expressed in a lineage- and differentiation stage-specific fashion. It is known that CD45 is expressed at low levels in hematopoietic progenitor cells and that this expression intensifies along with maturation.[20,21] Our FACS analysis data (Fig. 8) showing that CD45 expression was higher in $CD34^+/CD33^+$ cells than in $CD34^+/CD33^-$ cells confirmed this. Commitment to the erythroid lineage downregulates both CD45 and LSM-1 expression, whereas terminal differentiation to plasma cells in the lymphoid lineage and to granulocytes and monocytes in the myeloid lineage downregulates only LSM-1 expression, while high levels of CD45 expression are maintained.

It is speculated that LSM-1 (LPAP/CD45-AP) is associated with CD45 and transduces the signals from CD45.[15] At present, however, the mechanisms by which LSM-1 (LPAP/CD45-AP) mediates the signals from CD45 remain unknown. Since both hematopoietic progenitor cells expressed LSM-1 (low levels) and CD45 (intermediate to high levels), these cells may need the signals mediated by LSM-1 and CD45 for proliferation and/or differentiation. As terminal differentiation progresses, the roles of the signals mediated by these molecules in hematopoietic cell proliferation and/or differentiation may decrease and finally be lost. Erythroid lineage cells in particular will no longer need the signals mediated by LSM-1 and CD45

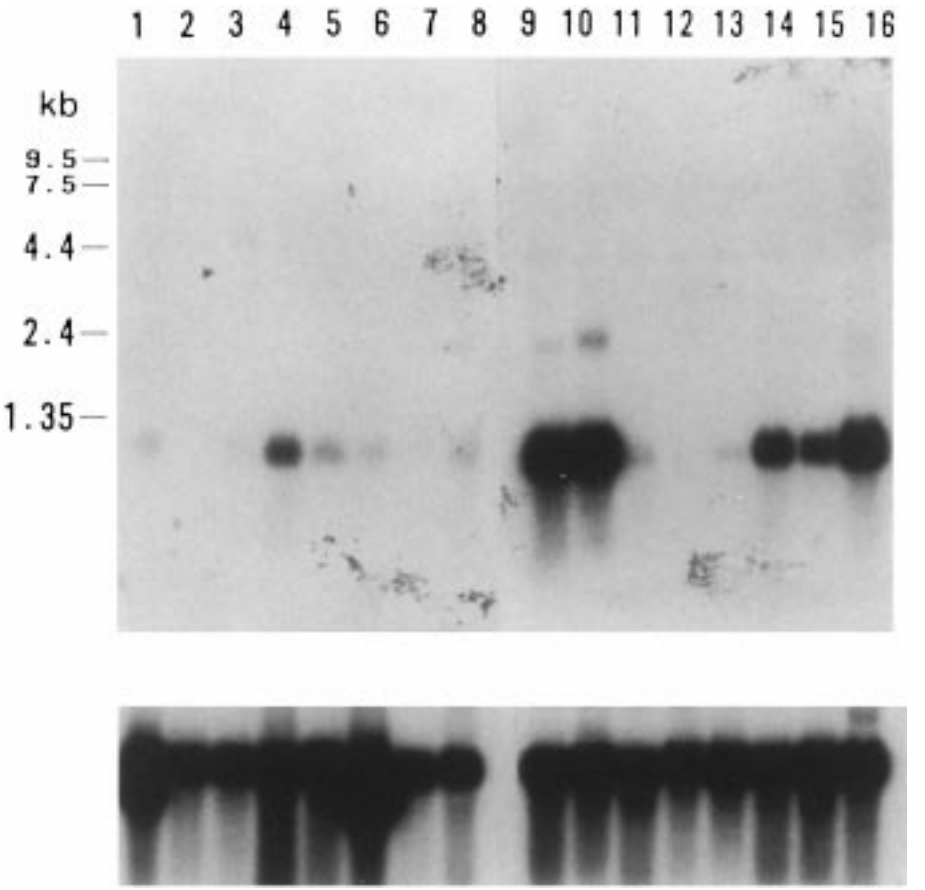

Fig. 9. Northern blot analysis for LSM-1 expression. RNA from various human tissues (Multiple Tissue Northern Blot Kits) was hybridized with ^{32}P-labeled hLSM-1 DNA probe. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas; lane 9, spleen; lane 10, thymus; lane 11, prostate; lane 12, testis; lane 13, ovary; lane 14, small intestine; lane 15, colon; lane 16, peripheral blood leukocyte.

for proliferation and/or differentiation at the earlier stages of differentiation. Whether or not CD45-mediated signals can be transduced in the absence of LSM-1 is of both interest and importance, so that further studies addressing this issue are awaited. To elucidate the roles of LSM-1 in the proliferation and/or differentiation of normal hematopoietic cells, the establishment of knockout and transgenic mice for LSM-1 gene is now in progress.

It was found that LSM-1 is not always needed for CD45 expression. The myeloma cell line (RPMI 8226), the monocyte-like cell line (U937), the erythroid cell line (K562), and plasma cells, granulocytes, and monocytes all expressed CD45 in the absence of LSM-1. Thus, CD45 can be expressed on cell surfaces in the absence of LSM-1. On the other hand, LPAP (LSM-1) protein could not be detected in a CD45-deficient Jurkat variant cell line.[15] However, this variant expressed similar amounts of LPAP mRNA as the CD45-positive counterparts. This indicates that the failure of cells to express normal amounts of LPAP (LSM-1) protein in the absence of CD45 results from post-transcriptional processes. A series of experiments suggested that LPAP (LSM-1) protein becomes synthesized in CD45-deficient cells but is then subjected to degradation and that portions of CD45 distinct from the phosphatase domains are required for the stabilization of LPAP (LSM-1).[15] Whether or not CD45-mediated signal can be transduced in the absence of the LSM-1 is of interest and of importance. Further study to address this issue is awaited.

LSM-1 was also expressed in various organs, including the small intestine and colon. Which kinds of cells of different organs express LSM-1 is of interest. Since the small intestine and colon, which strongly expressed LSM-1, have abundant lymphoid follicles, the lymphocytes in the follicles may express LSM-1. However, the possibility remains that cells other than lymphocytes express LSM-1. A histochemical study to determine which kinds of cells express LSM-1 in the small intestine and colon is now in progress.

ACKNOWLEDGMENTS

We thank Hiromi Takeuchi for her assistance in the preparation of the manuscript.

REFERENCES

1. Thomas ML, Lefrançois L: Differential expression of the leukocyte common antigen family. Immunol Today 9:320–326, 1988.
2. Thomas ML: The leukocyte common antigen family. Annu Rev Immunol 7:339–369, 1989.
3. Clark EA, Ledbetter JA: Leukocyte cell surface enzymology: CD45 (LCA, T200) is a protein tyrosine phosphatase. Immunol Today 10:225–228, 1989.
4. Trowbridge IS, Ostergaard HL, Johnson P: CD45: A leukocyte-specific member of the protein tyrosine phosphatase family. Biochim Biophys Acta 1095:46–56, 1991.
5. Koretzky GA: Role of the CD45 tyrosine phosphatase in signal transduction in the immune system. FASEB J 7:420–426, 1993.
6. Fujii Y, Okumura M, Inada K, Nakahara K, Matsuda H: CD45 isoform expression during T cell development in the thymus. Eur J Immunol 22:1843–1850, 1993.
7. Pingel JT, Thomas ML: Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. Cell 58:1055–1065, 1989.
8. Weaver CT, Pingel JT, Nelson JO, Thomas ML: $CD8^+$ T-cell clones deficient in the expression of the CD45 protein tyrosine phosphatase have impaired responses to T-cell receptor stimuli. Mol Cell Biol 11:4415–4422, 1991.
9. Schraven B, Kirchgessner H, Gaber B, Samstag Y, Meuer S: A functional complex is formed in human T lymphocytes between the protein tyrosine phosphatase CD45, the protein tyrosine kinase $p56^{lck}$ and pp32, a possible common substrate. Eur J Immunol 21:2469–2477, 1991.
10. Schraven B, Schirren A, Kirchgessner H, Siebert B, Meuer SC: Four $CD45/p56^{lck}$-associated phosphoproteins (pp29–pp32) undergo alterations in human T cell activation. Eur J Immunol 22:1857–1863, 1992.
11. Koretzky G, Kohmetscher M, Ross S: CD45-associated kinase activity requires lck but not T cell receptor expression in the Jurkat T cell line. J Biol Chem 268:8958–8964, 1993.
12. Mustelin T, Pessa-Morikawa T, Autero M, Gassmann M, Andersson LC, Gahmberg CG, Burn P: Regulation of the $p59^{fyn}$ protein tyrosine kinase by the CD45 phosphotyrosine phosphatase. Eur J Immunol 22:1173–1178, 1992.
13. Shimizu Y, Ogawa H, Oka Y, Mizuno R, Sakoda S, Kishimoto T, Sugiyama H: Isolation of a cDNA clone encoding a novel membrane protein expressed in lymphocytes. FEBS Lett 355:30–34, 1994.
14. Takeda A, Maizel AL, Kitamura K, Ohta T, Kimura S: Molecular cloning of the CD45-associated 30-kDa protein. J Biol Chem 269:2357–2360, 1994.
15. Schraven B, Schoenhaut D, Bruyns E, Koretzky G, Eckerskorn C, Wallich R, Kirchgessner H, Sakorafas P, Labkovsky B, Ratnofsky S, Meuer S: LPAP, a novel 32-kDa phosphoprotein that interacts with CD45 in human lymphocytes. J Biol Chem 269:29102–29111, 1994.
16. Inoue K, Wongsasant B, Akiyama T, Toyoshima K: Human c-fgr induces a monocyte-specific enzyme in NIH3T3 cells. Mol Cell Biol 11:6279–6285, 1991.
17. Okumura M, Fujii Y, Inada K, Nakahara K, Matsuda H: Both $CD45RA^+$ and $CD45RA^-$ subpopulations of $CD8^+$ T cells contain cells with high levels of lymphocyte function-associated antigen-1 expression, a phenotype of primed T cells. J Immunol 150:429–437, 1993.
18. Haruta N, Monden T, Morimoto H, Murotani M, Yagyu T, Nagaoka H, Shimano T, Mori T: Use of a rapid microwave fixation technique for immunocytochemical demonstration of tumor necrosis factor, interleukin-1α, and interleukin-1β in activated human peripheral mononuclear cells. Acta Histochem Cytochem 23:563–572, 1990.
19. Harada H, Kawano MM, Huang N, Harada Y, Iwato K, Tanabe O, Tanaka H, Sakai A, Asaoku H, Kuramoto A: Phenotypic difference of normal plasma cells from mature myeloma cells. Blood 81:2658–2663, 1993.
20. Shah VO, Civin CI, Loken MR: Flow cytometric analysis of human bone marrow. IV. Differential quantitative expressin of T-200 common leukocyte antigen during normal hemopoiesis. J Immunol 140:1861–1867, 1988.
21. Craig W, Poppema S, Little M-T, Dragowska W, Lansdorp PM: CD45 isoform expression on human haemopoietic cells at different stages of development. Br J Haematol 88:24–30, 1994.